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Immunology

Interleukin 13, a T-cell-derived cytokine that regulates human monocyte and B-cell function

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ABSTRACT We have isolated the human cDNA homologue of a mouse helper T-cell-specific cDNA sequence, called P600, from an activated human T-cell cDNA library. The human cDNA encodes a secreted, mainly unglycosylated, protein with a relative molecular mass of ≈10,000. We show that the human and mouse proteins cause extensive morphological changes to human monocytes with an associated up-regulation of major histocompatibility complex class II antigens and the low-affinity receptor for immunoglobulin E (FcεRII or CD23). In addition, they stimulate proliferation of human B cells that have been activated by anti-IgM antibodies or by anti-CD40 monoclonal antibodies presented by a mouse Ltk$^-$ cell line transfected with CDw32. Furthermore, the human protein induced considerable levels of IgM and IgG, but no IgA production, in cultures in which highly purified human surface IgD$^+$ or total B cells were cocultured with an activated CD4$^+$ T-cell clone. Based on these findings, we propose that this immunoregulatory protein be designated interleukin 13.

Key to the immune response elicited by particular antigens is the nature of the T-cell help that is invoked. Helper T cells react to antigen stimulation by producing soluble factors that regulate immune responses. Two subsets of helper T cells, designated Th1 and Th2, have been characterized in mouse and man (1, 2). The different spectra of soluble factors these cell types produce have been critical to their definition.

We have described previously a mouse cDNA that corresponds to an mRNA specifically produced by activated mouse Th2 cells (2, 3). The longest open reading frame (ORF) of this mRNA is 131 amino acids and shows no obvious homology to known proteins. The ORF encodes a polypeptide with a potential leader sequence, and it was postulated that the protein is a novel secreted immune regulatory factor (3). In this report we describe a cDNA encoding the human counterpart of the mouse protein and show that recombinant protein produced from the mouse and human cDNAs is capable of eliciting differentiation of human monocytes and differentiation and proliferation of human B cells. Due to the profound nature of these effects on various cell types of the immune system we will refer to these proteins as human interleukin 13 (hIL-13) and mouse IL-13 (mIL-13).¶

MATERIALS AND METHODS

Cells and Cell Lines. TF-1 cells are a human premyeloid cell line derived from a patient with erythroleukemia (4). Human peripheral blood mononuclear cells (PBMNC) were separated by a standard Ficoll–Hypaque gradient method. Human monocytes were isolated from human PBMNC by adherence to plastic.

Tonsillar B cells were prepared as described (5). Flow cytometric analysis following staining with fluorescein-labeled anti-CD19, -CD20, -CD14 (Becton Dickinson), -CD2, and -CD3 (Immunotech, Luminy, France) showed that the purity of this B-cell population was >95%.

Splenic B cells were purified from a normal human spleen, obtained from a transplant donor, by negative fluorescence-activated cell sorting (FACStar Plus, Becton Dickinson); splenocytes were stained with the following phycoerythrin-labeled antibodies (Becton Dickinson): anti-CD3, -CD4, -CD8, -CD14, -CD16, and -CD56 and the sorted cells were shown to be 99.9% CD20$^+$ when reanalyzed after staining with an anti-CD20 fluorescein isothiocyanate-labeled monoclonal antibody (FITC mAb) (Becton Dickinson). In some experiments surface IgD$^+$ B cells were isolated by two-color sorting after staining the splenocytes as outlined above, but with the addition of FITC-labeled anti-human IgD (Nordic, Lausanne, Switzerland). Reanalysis of these cells showed them to be >99% surface IgD$^+$.

Isolation of hIL-13 cDNA Clones. hIL-13 cDNA clones were identified by colony hybridization with a 400-bp *Pst* I/*Pvu* II DNA fragment, derived from the mIL-13 cDNA (3), as a probe. Hybridization was overnight at 42°C in 6× SSPE (1× SSPE = 150 mM NaCl/10 mM NaH_2PO_4/1 mM EDTA, pH 7.4), 20% formamide, and 0.1% SDS, with 1 mg of tRNA per ml. The filters were then washed three times at room temperature with 0.1× SSPE/0.05% SDS. The cDNA inserts of positive clones were subcloned into the *Bam*HI site of M13mp18 (6) and sequenced (Sequenase 2.0 kit, United States Biochemical).

RNA Blot Analysis. RNA (10 μg per lane) from activated or resting T cells was fractionated by electrophoresis through a 0.8% agarose/formaldehyde gel and blotted onto Nytran (Schleicher & Schuell). Hybridization was performed as described above.

Expression of Recombinant IL-13. Transfection of COS-7 cells and *in vitro* labeling with [^{35}S]methionine and [^{35}S]cysteine were performed as described (7).

IL-13 was also expressed as a fusion protein with glutathione-*S*-transferase using the pGEX-2T vector (Pharmacia). A DNA fragment encoding hIL-13 residues 24–132 was prepared by polymerase chain reaction (PCR) and cloned into the *Bam*HI/*Eco*RI site of the vector. A DNA fragment encoding mIL-13 residues 19–131 was also prepared and cloned as above. The hIL-13 and mIL-13 fusion proteins were expressed as insoluble aggregates in *Escherichia coli*, extracted by centrifugation, solubilized, and subjected to a renaturation step (8). The refolded IL-13 was cleaved from

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Abbreviations: IL, interleukin; hIL-13, human IL-13; mIL-13, mouse IL-13; MHC, major histocompatibility complex; mAb, monoclonal antibody; ORF, open reading frame; PBMNC, peripheral blood mononuclear cells.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. L06801).

the fusion partner by thrombin (9) and purified by cation-exchange [S-Sepharose fast-performance liquid chromatography (FPLC), Pharmacia] and gel filtration (Sephacryl S-200 FPLC, Pharmacia) chromatography. Endotoxin (determined by the *Limulus* amebocyte lysate assay, Whittaker Bioproducts) was typically <1 endotoxin unit/ml.

Assays for IL-13. TF-1 cell proliferation assays using 2×10^4 cells per well were performed as described (7). Cell proliferation was determined by colorimetric assay (10).

B-cell proliferation assays were performed using purified human tonsillar B cells cultured in Iscove's medium enriched with 50 μg of human transferrin per ml, 5 μg of bovine insulin per ml, 0.5% bovine serum albumin, and 5% fetal calf serum. Human B-cell proliferation in the presence of costimulatory anti-IgM antibodies (10 μg/ml, Bio-Rad) was measured by plating 1×10^5 cells per well in the presence of mIL-13 (100 ng/ml), hIL-13 (100 ng/ml), or human interleukin 4 (IL-4) (50 units/ml). These concentrations of hIL-13 and mIL-13 were determined to give maximal B-cell stimulation (unpublished data).

The influence of anti-CD40 on human B-cell proliferation was determined by coculturing 10^5 human B cells in the presence of the anti-CD40 mAb 89 presented by a mouse Ltk$^-$ cell line (seeded at 10^4 cells per ml) stably expressing CDw32 (11) with the addition of mIL-13 (100 ng/ml), hIL-13 (100 ng/ml) or human IL-4 (50 units/ml). The incorporation of [^{3}H]thymidine was determined after 72 hr for the anti-IgM stimulation or after 6 days in the CD40 system.

For induction of immunoglobulin production highly purified surface IgD$^+$ or total B cells were cultured at 5×10^3 cells per well in the presence of the CD4$^+$ T-cell clone B21 (10^4 cells per well) and recombinant IL-2 (10 units/ml). IL-13 or IL-4 was added at 30 ng/ml. After 14 days the supernatants were harvested for determination of their immunoglobulin content (IgM, IgG, and IgA) by enzyme-linked immunosorbant assay (ELISA) as described (12). The sensitivities of the IgM, IgG, and IgA ELISAs were 0.5–1 ng/ml.

RESULTS

hIL-13 cDNA Cloning. cDNA libraries constructed from the mRNAs of a number of T-cell clones were screened using a mouse P600 cDNA probe. Positive hybridization (representing 0.3% of all clones) was initially observed with cDNAs derived from the T-cell clone B21. However, the longest hIL-13 cDNA clone identified from the B21 library was found to lack sequences corresponding to the 30 N-terminal amino acids of the mouse protein. Full-length cDNA clones were subsequently isolated from a cDNA library derived from a Con A-activated CD8$^+$ T-cell clone, A10. The A10-derived cDNA contained a single ORF encoding a polypeptide of 132 amino acids. Comparison with a proposed consensus sequence for the processing of signal peptides (13) predicted Ser-19 as the N terminus of the mature human protein. The

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h M A L L L T T V I A L T C L G G F A S P G P V P P S         26
m M A L W V T A V L A L A C L G G L A A P G P V P R S V S

      T A L R E L I E E L V N I T Q N Q K A P L C N G S M     52
L P L T L K E L I E E L S N I T Q D E T   P L C N G S M

V W S I N L T A G M Y C A A L E S L I N V S G C S A I E       80
V W S V D L A A G G F C V A L D S L T N I S N C N A I Y

K T Q R M L S G F C P H K V S A G Q F S S L H V R D T K      110
R T Q R I L H G L C N R K A P T T V   S S L P     D T K

I E V A Q F V K D L L L H L K K L F R E G R F N 132
I E V A H F I T K L L S Y T K Q L F R H G P F
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FIG. 1. Amino acid sequence alignment of hIL-13 and mIL-13. Identical amino acids are boxed. The predicted N-linked glycosylation sites are underlined. The single-letter code for amino acids is used.

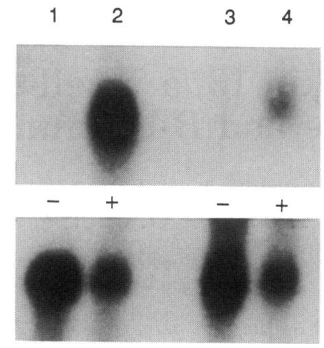

FIG. 2. Northern blot analysis of RNA from resting and activated T cells. Lanes 1 and 2, RNA prepared from resting and induced B21 cells (14), respectively; lanes 3 and 4, RNA prepared from resting (−) and induced (+) human ES228 cells (15), respectively, and probed with either hIL-13 cDNA (top) or β-actin cDNA (bottom).

cDNAs isolated from the B21 and A10 libraries differed in that the clone from the A10 library encoded an additional glutamine residue at position 98 (Fig. 1). PCR analysis of the two libraries revealed that both mRNA forms were made by both cell types (data not shown). Investigation of the hIL-13 gene suggests that this amino acid results from alternative splicing at an intron–exon boundary (unpublished data). hIL-13 displayed 66% nucleotide sequence identity over the coding region and 58% amino acid sequence identity to mIL-13 (Fig. 1). All five of the cysteine residues, including one in the putative leader sequence, were conserved. hIL-13 contained one extra potential N-linked glycosylation site in addition to three conserved sites (Fig. 1).

RNA blot analysis identified the presence of a single 1.3-kb hIL-13 mRNA species from activated T-cell clones that was absent from preparations from unactivated cells (Fig. 2). Northern blot analysis of RNA from several other human tissues, including heart, brain, placenta, lung, liver, and skeletal muscle (Clontech), was negative when probed with hIL-13 cDNA, but the control β-actin cDNA probe gave positive signals on all tissues (data not shown).

Expression of Recombinant hIL-13. COS-7 cells were transfected with an expression plasmid containing the full-length hIL-13 cDNA and metabolically labeled *in vitro* with [^{35}S]methionine and [^{35}S]cysteine. The transfected cells were grown in the presence and absence of tunicamycin (5 μg/ml; Sigma),

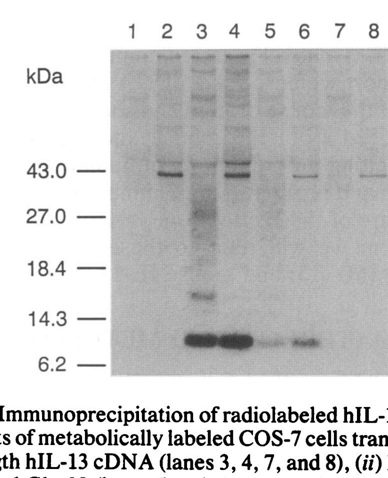

FIG. 3. Immunoprecipitation of radiolabeled hIL-13 protein from supernatants of metabolically labeled COS-7 cells transfected with (*i*) the full-length hIL-13 cDNA (lanes 3, 4, 7, and 8), (*ii*) hIL-13 without the additional Gln-98 (lanes 5 and 6), or (*iii*) the expression plasmid alone (lanes 1 and 2). Tunicamycin was included in cultures shown in lanes 2, 4, 6, and 8. Culture supernatants were collected and immunoprecipitated (16) using either rat polyclonal antiserum (lanes 1–6), raised as described (16) against *E. coli*-produced hIL-13, or normal rat serum (lanes 7 and 8). Immunoprecipitated material was analyzed using 12.5% SDS/PAGE and autoradiography (16).

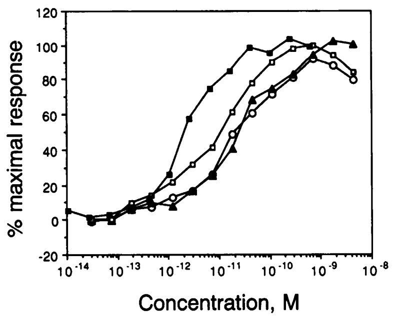

FIG. 4. Human TF-1 cell proliferation induced by IL-13. □, hIL-13; ○, hIL-13 (without Gln-98); ▲, mIL-13; ■, hIL-4.

an inhibitor of N-linked glycosylation (7). Immunoprecipitation was performed on the COS-7 cell supernatants using serum from rats immunized with recombinant hIL-13. [^{35}S]Methionine/cysteine labeled supernatants from mock-transfected cells were used as a negative control. Analysis of the immunoprecipitates using SDS/PAGE demonstrated that the majority of hIL-13 is secreted as a protein of ≈10 kDa, with little or no N-linked carbohydrate (Fig. 3). The two other minor, higher molecular mass, hIL-13 protein species are probably the result of differential glycosylation (Fig. 3).

Recombinant hIL-13 and mIL-13 proteins were also expressed in *E. coli*, as in-frame fusions with glutathione-*S*-transferase, and purified to homogeneity after cleavage with thrombin. The cleaved material eluted from a gel filtration column as monomeric protein of ≈10 kDa (see *Materials and Methods*).

Biological Activity of IL-13. Recombinant hIL-13 and mIL-13, produced by *E. coli*, were tested in various *in vitro* cellular proliferation assays. The human premyeloid cell line TF-1 proliferated in response to hIL-13 and mIL-13, whereas no effect was observed on various cell lines that respond to other T-cell-derived growth factors, including clone K (17), KD83 (provided by V. Ghanta, University of Alabama), NLO (provided by D. Rennick, DNAX), Ba/F3 (18), MC/9 (19), and M1 (20) (data not shown). hIL-13 (with or without the presence of Gln-98) and mIL-13 appeared to have the same specific activity on TF-1 cells (Fig. 4). Furthermore, mIL-13 and hIL-13 expressed by COS-7 cells were biologically active on TF-1 cells, and equivalent activity was present in supernatants from COS-7 cells cultured in the presence of tunicamycin (data not shown). This verified that N-linked glycosylation does not affect the biological activity of recombinant IL-13; however it remains to be determined what effect N-linked and O-linked glycosylation have on the activity of native IL-13.

IL-13 Induces Differentiation of Human Monocytes. Following our observation that IL-13 elicits cellular proliferation in at least one cultured myeloid cell type, the responses of a number of primary immune cell types to IL-13 were examined. The adherent fraction of human PBMNC containing predominantly human monocytes showed marked changes in morphology and cell surface phenotype when cultured in the presence of mIL-13 or hIL-13. Cells grown in the absence of this factor began to round up and detach from the substrate within 5 days (Fig. 5*A*), whereas those treated with IL-13 flattened and formed extensive processes and lamellar sheets (Fig. 5*B*). Cellular aggregates also formed in the presence of IL-13 (Fig. 5*B*). Furthermore, monocytes cultured in IL-13 showed increased survival time in culture and remained viable for >30 days. Flow cytometric analysis revealed that monocytes cultured in IL-13 also displayed significant modulation of certain cell surface markers. The IL-13-treated

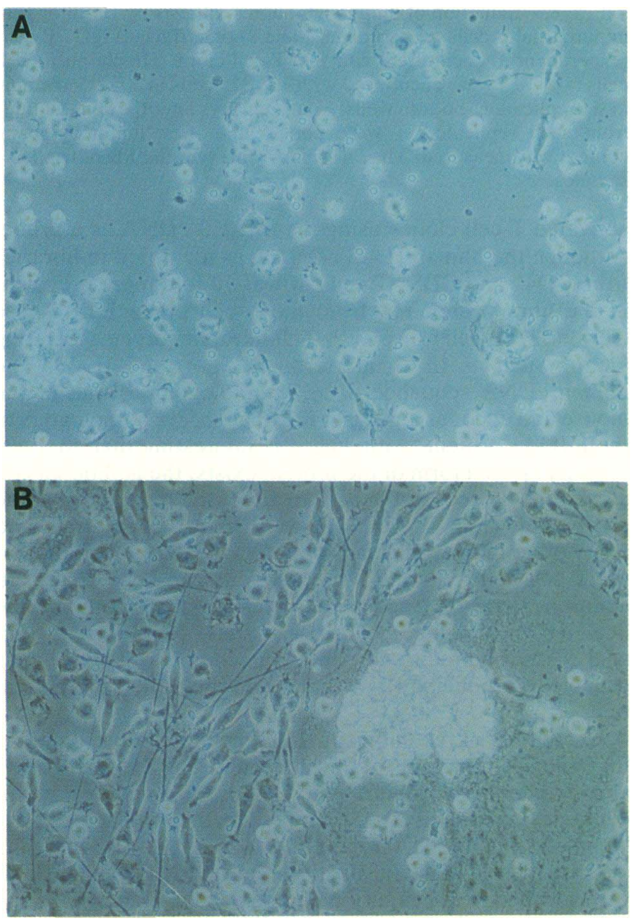

FIG. 5. Culture in IL-13 causes marked morphological changes to adherent monocytes. Adherent cells were cultured in either the absence (*A*) or the presence (*B*) of IL-13 (30 ng/ml) for 5 days.

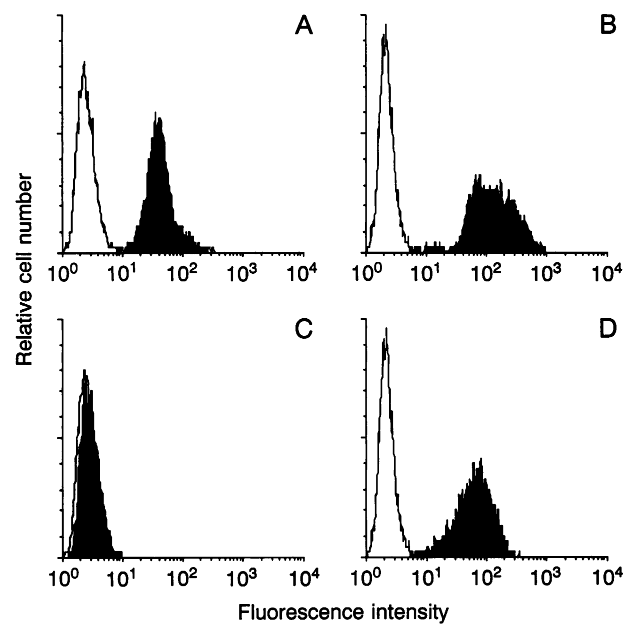

FIG. 6. Flow cytometric analysis shows that IL-13 up-regulates expression of MHC class II and CD23 (FcεRII) on PBMNC. Adherent PBMNC were cultured in the absence (*A* and *C*) or presence (*B* and *D*) of IL-13 (30 ng/ml). After 5 days the cells were collected and analyzed for expression of MHC class II antigens (*A* and *B*) using mAb pdV5.2 (21) and CD23 (*C* and *D*) using mAb gp25 (22) by indirect immunofluorescence as described (23).

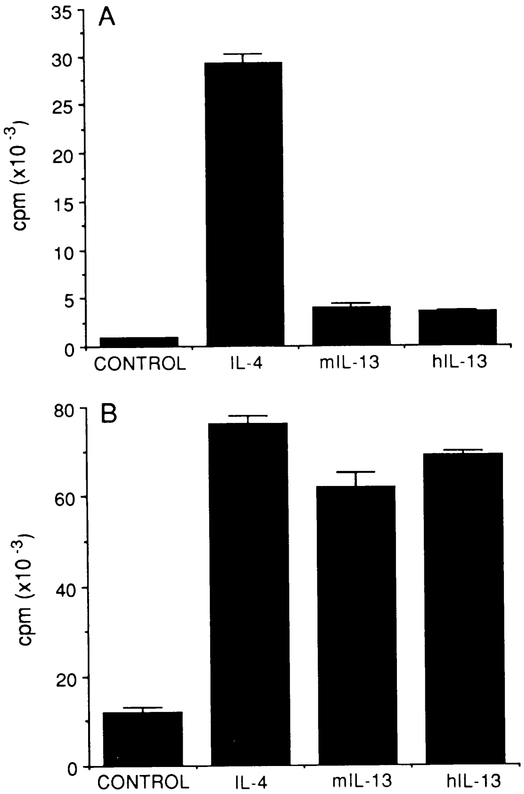

FIG. 7. IL-13 induces the proliferation of activated B lymphocytes. Purified human tonsillar B cells activated with anti-IgM (5×10^5 cells per ml) (*A*) or in the CD40 system (10^5 cells per ml) (*B*) were cultured without or with IL-4 (50 units/ml) or IL-13 (100 ng/ml) for 3 and 6 days in the respective activation mode. Each value represents the mean ± SD of triplicate samples. The data are representative of three experiments using mIL-13 and two experiments using hIL-13.

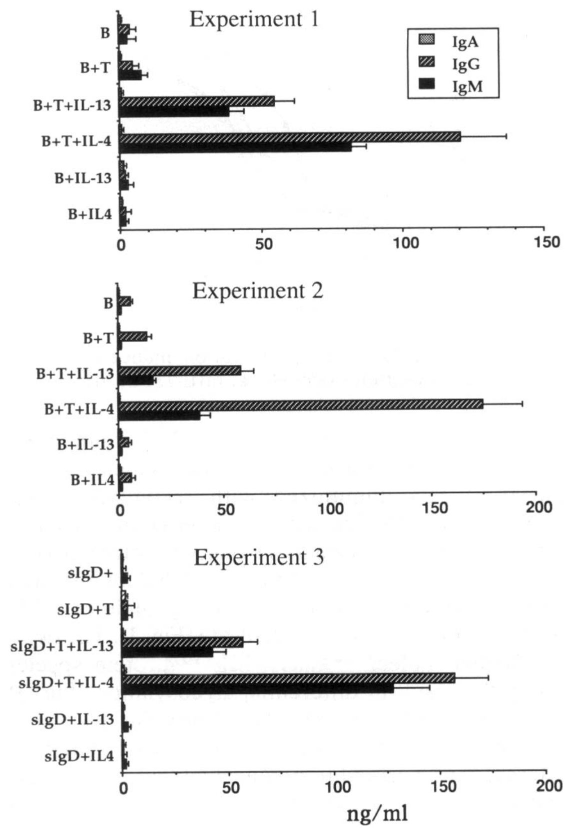

FIG. 8. Induction of immunoglobulin synthesis by IL-13 or IL-4 in pure total B cells (experiments 1 and 2) or surface(s) IgD$^+$ B cells (experiment 3) cocultured with B21 T-cell clone. Highly purified B cells (5×10^3) were cocultured with 10^4 B21 cells in Yssel's medium in six replicate U-bottom wells. Fourteen days later the supernatants from each replicate set were pooled and their immunoglobulin content was measured. The error bars represent the SD of triplicate samples.

cells displayed increased expression of major histocompatibility complex (MHC) class II antigens, and expression of the low-affinity receptor for IgE (FcϵRII, CD23) was induced (Fig. 6).

Induction of B-Cell Proliferation and Differentiation by IL-13. Anti-IgM-activated human tonsillar B cells displayed enhanced DNA synthesis when cultured in the presence of either hIL-13 or mIL-13 (Fig. 7). However, this proliferation was weak in comparison to that induced by IL-4 in the same assay system. In contrast, when cultured in the presence of anti-CD40 and IL-13, the B cells showed proliferation that was comparable to that induced by IL-4 (Fig. 7).

We also found that hIL-13, added to cultures of highly purified B cells in the presence of the activated CD4$^+$ T-cell clone B21, induced IgM and IgG synthesis. In general, a 4- to 12-fold increase in the production of these immunoglobulin isotypes was observed (Fig. 8 experiments 1 and 2). However, no measurable levels of IgA could be detected (Fig. 8). In other experiments in which surface IgD$^+$ B cells were used similar results were obtained (for a representative experiment see Fig. 8 experiment 3), confirming the notion that IL-13 induces B-cell differentiation and isotype switching. This also rules out the possibility that the observed immunoglobulin production reflects the B-cell growth-promoting effects of IL-13. Although IL-4 caused similar effects, it was generally more potent in stimulating immunoglobulin production than IL-13.

DISCUSSION

We have cloned a cDNA encoding a human cytokine called IL-13. cDNA clones were isolated from cDNA libraries from activated T-cell clones using the mIL-13 cDNA as a probe. Although the human IL-13 cDNA was identified in libraries from CD4$^+$ and CD8$^+$ T cells, it was 10-fold more prevalent in the library prepared from the T-cell clone B21. These CD4$^+$ B21 T cells display a Th0-like phenotype, secreting factors such as IL-2, interferon γ, IL-4, and IL-10. The human cDNA sequence is 66% homologous to that of the mouse and encodes a polypeptide that is 58% homologous to the mIL-13 protein. Although it is interesting that an alternatively spliced form of the protein exists, the additional Gln at position 98 does not appear to affect the bioactivity of the polypeptide (Fig. 4). hIL-13, like its mouse counterpart and many other immunoregulatory factors, requires activation of the T cell before its mRNA transcripts can be detected. In addition, IL-13 message is not widespread and could not be detected in a number of other tissues. However, further analysis is required to determine the complete range of cells capable of producing IL-13 and their requirement for activation.

Expression of recombinant IL-13 facilitated investigations for biological functions of this molecule. The TF-1 cell line is already known to proliferate in response to a wide variety of cytokines, including IL-3, granulocyte/macrophage colony-stimulating factor, erythropoietin, IL-5, and IL-4. Our results indicate that this cell line also responds to hIL-13 and mIL-13 with no species specificity being detectable (Fig. 4).

IL-13 also had a profound effect on primary immune cells, inducing immunoglobulin production and proliferation of B cells and the differentiation of cells of the monocytic lineage. The biological activities reported here indicate that IL-13 and a key Th2-derived cytokine IL-4 share certain functional characteristics, including the induction of the macrophage-like dendritic cell morphology (24) with associated CD23 expression on monocytes (25) and stimulation of immunoglobulin synthesis by B cells (26). Although IL-13 was less potent than IL-4 in enhancing the proliferation of B cells activated through their antigen receptor, the growth-promoting effects of the two cytokines were comparable for anti-CD40-stimulated B lymphocytes.

hIL-13 induced considerable levels of IgM and IgG, but no IgA, in cultures of highly purified surface IgD^+ or total B cells in the presence of an activated $CD4^+$ T-cell clone. IL-13 failed to induce immunoglobulin secretion in the absence of the $CD4^+$ T cells, indicating that, in addition to IL-13, costimulatory signals delivered by a $CD4^+$ T helper clone are required for productive IgM and IgG synthesis (26). IL-4 tested in parallel with IL-13 in these cultures had similar effects. The observation that IL-13 also induced IgM and IgG production by purified naive surface IgD^+ B cells in the presence of T-cell clones indicates that IL-13 induces B-cell differentiation and isotype switching, since IL-13 does not act on T cells or preactivated T cells (unpublished data). These results are compatible with more recent studies in which we have shown that IL-13 also directs human B cells to switch to IgE synthesis (27). Like IL-13, IL-4 stimulated IgM and IgG synthesis. Although IL-4 seemed in general to be more potent than IL-13, the levels of IgM and IgG produced were in the same range. IL-4, like IL-13, also failed to induce IgA synthesis, which is compatible with previous observations indicating that IL-4 specifically suppresses IgA production (26). Though it is clear that IL-13 and IL-4 share certain biological responses, it is also true that they can act distinctly. It is thus tempting to speculate that human B-cell subpopulations may respond differentially to these cytokines at various stages of their development. Further studies are necessary to elucidate how IL-13 interacts with other cytokines and how it may act in an ongoing immune response.

We expect that further investigation will reveal additional biological activities for IL-13. Such investigation, together with careful analyses of the distribution of cell types producing IL-13, should help clarify its precise biological role.

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